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U.S. ARMY PUBLIC HEALTH COMMAND (Provisional)

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ENVIRONMENTAL HEALTH ASSESSMENT FOR WORK UNIT RM 08-03 AMMONIUM PERCHLORATE ALTERNATIVES (IONIC LIQUIDS) TOXICOLOGY REPORT NO. 87-XE-0BMEa-10

Preventive Medicine Surveys: 40-5f1

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14. ABSTRACT

This Environmental Health Assessment is part of an ongoing effort to assess the human health and environmental impact of items of Army materiel during the development process. The objective of this program is to reduce the environmental and human health impact of newly-developed Army end items, with concomitant reductions in overall cost, without sacrificing performance. This report addresses human health and environmental impacts of ionic liquids being investigated as potential replacements for ammonium perchlorate and hydrazine propellants.

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Ionic liquids, ammonium perchlorate replacements, hydrazine replacements

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1 Summary

1.1 Purpose

To provide environmental and occupational health information on new or replacement energetic compounds for Army use in the research, development, testing, and evaluation (RDT&E) of alternatives under the Environmental Quality Technology (EQT) program. This information is necessary for work unit program evaluation.

Research, development, testing, training, and use of substances potentially less hazardous to human health and the environment is vital to the readiness of the U.S. Army. Safeguarding the health of Soldiers, civilians, and the environment requires an assessment of alternatives before they are fielded. Continuous assessments begun early in the RDT&E process can save significant time and effort during RDT&E, as well as over the life cycle of the items developed. Residues of pyrotechnics, propellants, explosives, and incendiaries have been found in soil, air, surface, and ground water samples, creating environmental problems and interfering with training activities.

The U.S. Army EQT Ordnance Environmental Program is dedicated to finding replacements for substances causing environmental and/or occupational risks to health. As part of this program, each work unit is evaluated for environmental and occupational health impacts. The purpose of this work unit is to identify possible propellant chemical mixtures to replace hydrazine and/or ammonium perchlorate (AP) in rocket/missile propellant formulations. The replacement mixture should be of equal or greater efficiency but also be more conducive to human health and environmental quality than the traditional compounds. Hydrazine is both hazardous to handle and a human and environmental health hazard. While AP is traditionally the oxidizer of choice for propellant formulations, it is known to compete with iodide for uptake by the thyroid gland and at high concentrations suppresses thyroid production. Pregnant and nursing women, infants, and young children are considered a sensitive subpopulation because of potential neurodevelopmental impacts associated with low thyroid hormone levels.

The substances considered in this report are commercially available and can readily be incorporated into existing propellant systems. Research into the performance capabilities of these compounds is being conducted by the U.S. Army Aviation and Missile Research, Development and Engineering Command (Dr. Gregory Drake, Principal Investigator).

1.2 Conclusions

The ionic liquids considered in this work unit as replacements for hydrazine are less toxic than hydrazine and appear to pose less of a threat to human health than ammonium perchlorate, but lack of experimental data is a major concern. The high water solubility of ionic liquids, coupled with their low affinity for organic carbon, make it likely they will transport through the environment if they enter ground water, and humans may be exposed via drinking water. Due to a lack of experimental data, many of the conclusions of this report are based upon modeling using Quantitative Structure Activity Relationships (QSAR). Based upon modeling, some of the substances under consideration may be developmental toxicants. Toxicological testing is recommended should these compounds be selected for further development.

1.3 Recommendations

In view of the general lack of experimental data, collection of basic information with respect to both physical/chemical and toxicological properties should be undertaken for compounds selected for further development. Physical/chemical properties that should be experimentally confirmed or evaluated include water solubility, octanol-water partition coefficients, and biodegradation/ persistence.

A battery of *in vitro* tests (see Table 9) is also recommended to evaluate/validate the Quantitative Structure-Activity Relationship (QSAR) predictions that these compounds are developmental or reproductive toxicants, mutagenic, or carcinogenic. Depending upon the outcome of these initial *in vitro* tests, additional testing may be required.

2 References

See Appendix A for a listing of references used in this report.

3 Authority

This Environmental Health Assessment addresses, in part, the environmental safety and occupational health (ESOH) requirements outlined in Army Regulation (AR) 200-1; AR 40-5; and AR 70-1; Department of Defense Instruction (DoDI) 4715.4; and Army Environmental Research and Technology Assessment (AERTA, 2009) requirement PP-3-02-04, *Compliant Ordnance Lifecycle for Readiness of the Transformation and Objective Forces*. This assessment was performed as part of an on-going effort by the U.S. Army EQT OEP to reduce or eliminate the environmental impact of new chemical formulations proposed for use in weapon systems or platforms. This program is supported by the U.S. Army Research, Development and Engineering Command (USARDECOM) Environmental Acquisition Logistics & Sustainment Program (EALSP); Mr. Erik Hangeland, Director, via MIPR 0BDAT4D100.

4 Background

Current regulations require assessment of human health and environmental effects arising from exposure to chemical substances in soil, surface water, and ground water. Applied after an item has been fielded, these assessments can reveal the existence of adverse environmental and human health effects that can effect mission requirements and result in substantial costs and

adverse health consequences. It is most efficient to begin the evaluation of exposure, effects, and environmental transport of military-related compounds/substances early in the research, development, testing, and evaluation (RDT&E) process in order to avoid unnecessary costs, conserve physical resources, and sustain the health of those potentially exposed.

In an effort to support this preventive approach, the U.S. Army Public Health Command (Provisional) (USAPHC (Prov)), formerly the U.S. Army Center for Health Promotion and Preventive Medicine (USACHPPM), has been directed to create a phased evaluation process to reduce adverse ESOH effects impacting readiness, training, development, and potential remediation to sustain the environmental integrity of testing and training ranges. Assessing human health and environmental impact is an ongoing effort, and this report represents the status of information available for this work unit as of the date of publication. These replacements are being developed to reduce adverse ESOH effects for substances that have been found to affect readiness and costs associated with training. The Principal Investigator for the research effort is Dr. Gregory Drake, U.S. Army Aviation and Missile Research, Development and Engineering Center, Redstone Arsenal, Huntsville, Alabama.

5 Statement of Problem

Hydrazine is a colorless, oily liquid that fumes in air. It has been used as a liquid propellant in military missile systems since the end of World War II; its most recent applications include use as satellite on-board maneuvering fuel and as the propellant in the Lance missile system. Hydrazine is extremely dangerous to handle, producing severe, penetrating burns. Occupational exposure to hydrazine has been noted to produce systemic problems in humans, to include lung and liver damage, conjunctivitis, tremors, lethargy, long-term neurobehavioral impairment and can cause death. Hydrazine is a potent mutagen and is classified as a possible human carcinogen, based upon inadequate evidence in humans but sufficient evidence in animals (HSDB, 2009).

Ammonium perchlorate is currently used as an oxidizer in many rocket and missile propellant formulations. Perchlorate anion (or ClO_4^-) is a soluble ion that is environmentally persistent and mobile, especially in surface waters. A known toxicant, with well understood modes and mechanisms of action, perchlorate is easily absorbed through ingestion and inhalation. The perchlorate anion has numerous environmental and human health regulatory concerns. Perchlorate competes with iodine and inhibits the production of thyroid hormones in the human body. While individuals who consume recommended quantities of iodine in their diet appear not to be affected, a subpopulation of persons who are iodine-deficient (nursing mothers and infants) may be at risk of thyroid hormone deficiency. Thyroid hormone deficiency has been shown in the developing rat model to result in impaired neurological development (NRC, 2005).

6 Methods

In order to determine the human health and ecological impacts of compounds employed in these formulations, it is necessary to correctly and unambiguously identify each compound and to determine its physical, chemical, and toxicological properties. The primary means of identification employed for each compound in this program is its Chemical Abstracts Service Registry Number (CASRN) (Table 1). While all compounds do not necessarily have a single CASRN, the CASRN is an unambiguous way of accessing information for chemical substances. The CASRN is readily used as a keyword for searching online databases and is often cross-referenced with both systematic and trivial (i.e., "common") names for chemical substances. In some cases, synonyms

and trade names are also used to identify structures.

Basic physical and chemical properties are usually determined by consulting tertiary sources when such information is available. The properties necessary to assess fate and transport in the environment (FTE) include—

- Molecular weight (MW).
- Henry's law constant (K_H).
- Octanol-water partition coefficient (log K_{OW}).
- Water solubility
- Boiling point (bp).
- Organic carbon partition coefficient (log K_{OC}).
- Vapor pressure (vp).

Available information on combustion, explosion, and thermal decomposition products is also collected if available. Toxicological information needed to estimate potential human health risks includes reported toxicity effects of acute, subacute, subchronic, and chronic exposures; potential for mutagenesis and carcinogenesis; and mode(s) and mechanisms of toxicity. Toxicological information is derived directly from primary sources whenever possible.

Hardcopy sources used in this search included publications from the U.S. Department of Health and Human Services (DHHS), Agency for Toxic Substances and Disease Registry (ATSDR), and *The Merck Index* (O'Neil, 2006). The Chemical Propulsion Information Agency's (CPIA), *Hazards of Chemical Rockets and Propellants* (CPIA, 1985), and the U.S. Environmental Protection Agency's (USEPA) *Drinking Water Health Advisory: Munitions* (USEPA, 1992), were also consulted. Commercial suppliers are sometimes contacted for results of in-house research that may not appear in the open literature.

Online sources include the Defense Technical Information Center (DTIC©) and U.S. National Library of Medicine's Toxicology Data Network (TOXNET®) that provides access to information from the National Institutes of Health and the USEPA. TOXNET® is a suite of individual databases including ChemIDPlus (CIDPL) Lite® and ChemIDPlus® Advanced (i.e., chemical and registration numbers, and chemical identification and structure, searches respectively), Hazardous Substances Data Bank (HSDB®), Chemical Carcinogenesis Research Information System (CCRIS), Developmental and Reproductive Toxicology (DART/ETIC), Directory of Information Resources Online (DIRLINE®), Genetic Toxicology (GENE-TOX), Haz-Map (database linking chemicals, jobs and diseases), Household Products (potential health effects of chemicals in common household products), Integrated Risk Information System (IRIS), International Toxicity Estimates for Risk (ITER), Toxicology Information Online (TOXLINE®), Toxic Release Inventory (TRI), and Lactation Database (LactMed) (database of drugs and other chemicals to which breastfeeding mothers may be exposed). Primary sources are identified and retrieved using PubMed®, the Ovid® Technologies Journals, and the EBSCOhost® Research Database. (TOXNET®, ChemIDPlusLite®, ChemIDPlus®, HSDB®, DIRLINE®, TOXLINE®, PubMed®, are registered trademarks U.S. National Library of Medicine; OVID®, is a registered trademark of Ovid Technologies, Inc.; and EBSCOhost® is a

registered trademark of EBSCO Industries, Inc.) Some properties are not measured experimentally but are estimated by QSAR models such as TOPKAT® or EPI Suite™ used to predict physical, chemical and toxicological properties of compounds. (TOPKAT is a registered trademark of Accelrys, Inc., EPI Suite is a trademark of USEPA and Syracuse Research Corporation.)

Persistence, bioaccumulation, human health toxicity, and ecotoxicity were assigned to general categories of risk (e.g., low, moderate, and high) using criteria modified from Howe et al. (2006). Table 2 describes the criteria used in the categorization, though the relative proportions of each substance were also factored into the final assessment.

Table 1. Compound Identification Information

Compound	Chemical Symbol	CASRN
1-ethyl-3-methylimidazolium dicyanamide	[EMIM][DCA]	370865-89-7
1-butyl-3-methylimidazolium dicyanamide	[BMIM][DCA]	448245-52-1
1-butyl-1-methylpyrrodlidinium dicyanamide	[BMP][DCA]	370865-80-8
Dicyanamide (Dicyanoamide, sodium salt)	[DCA]	504-66-5/ 1934-75-4
1,2,4-trimethyl-1,4-diazabicyclo[2.2.2]octane dinitrate	None assigned	Not found
tris-(2-nitratoethyl)methylammonium nitrate	None assigned	Not found

7 Results

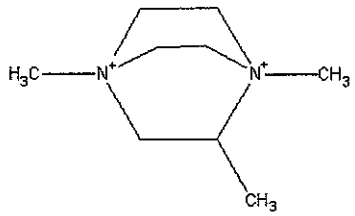
7.1 Physical and Chemical Properties

Physical and chemical properties are summarized in Table 4. Quantitative toxicological data is tabulated in Table 5. Data for properties that could not be found are indicated by "nd" (no data). In some cases the property named is not applicable ("n/a") to the substance being described.

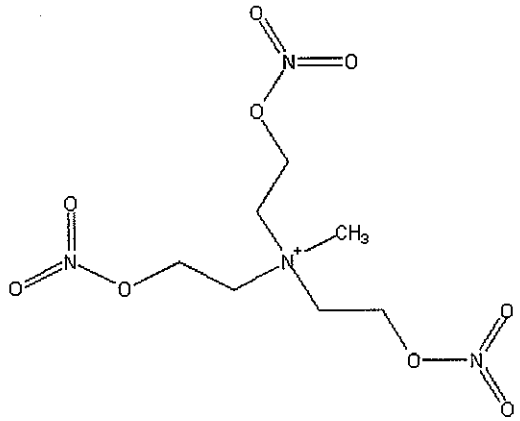
7.2 Human Health and Environmental Toxicology Summaries

Summaries of human health and environmental toxicology for each of the formula components are presented in Tables 6 and 7, respectively. Each characterization is generally based on the criteria set forth in Table 2. The final risk characterization also incorporates assessment of the uncertainty associated with available data, the amount of each compound present in the formulation, and the nature of potential exposure associated with use of the end item.

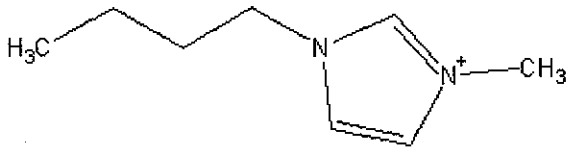
1-ethyl-3-methylimidazolium cation



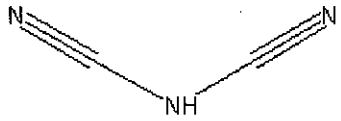
1,2,4-trimethyl-1,4-diazabicyclo [2.2.2] octane (dinitrate)



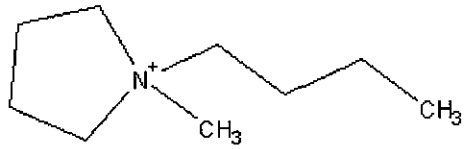
Tris-(2-nitratoethyl)methylammonium cation



1-butyl-3-methylimidazolium cation



Dicyanamide anion



1-butyl-1-methylpyrridolinium cation

Figure 1. Chemical Structures of Compounds Under Consideration

Table 2. Categorization Criteria used in the Development of Environmental Safety and Occupational Health Severity (Howe et al., 2006)

	LOW	MODERATE	HIGH
PERSISTENCE	Readily biodegrades (<28 days)	Degradation ½ life: water <40 days , soil <120 days	Degradation ½ life: water >40 days soil > 120 days
TRANSPORT	Water sol. < 10 mg/L log K_{OC} > 2.0	Water sol. 10-1000 mg/L log K_{OC} 2.0-1.0	Water sol. > 1000 mg/L log Koc <1.0
BIOACCUMULATION	log K_{OW} <3.0	log K_{OW} 3.0-4.5	log K_{OW} >4.5
TOXICITY	No evidence of carcinogenicity/ mutagenicity; Subchronic LOAEL > 200 mg/kg-d	Mixed evidence for carcinogenicity/mutagenicity (B2, 2); Subchronic LOAEL 5-200 mg/kg-d	Positive corroborative evidence for carcinogenicity /mutagenicity; LOAEL < 5 mg/kg-d
ECOTOXICITY	Acute $LC_{50}LD_{50}$ >1 mg/L or 1500 mg/kg; Subchronic EC_{50} >100 µg/L or LOAEL >100 mg/kg-d	Acute $LC_{50}LD_{50}$ 1-0.1 mg/L or 1500-150 mg/kg; Subchronic EC_{50} 100-10 µg/L or LOAEL – 10-100 mg/kg-d	Acute LC_{50},LD_{50}<100 µg/L or <150 mg/kg; Subchronic LOAEL <10 mg/kg-d

Legend:
mg/L - milligrams per liter
LC_{50} – concentration expected to result in 50% lethality to a population of test animals
LD_{50} - lethal dose 50 median concentration of a toxicant that will kill 50% of the test animals within a designated period
mg/kg-d - milligram per kilogram per day; mg/kg – milligram per kilogram
µg/L - microgram per liter
LOAEL – lowest-observed adverse effect level
EC_{50} – exposure concentration resulting in mortality to 50% of a population

7.3 1-Ethyl-3-methylimidazolium dicyanamide, [EMIM][DCA]*

Virtually no experimental toxicological data are available for this or structurally similar compounds. The information below pertains primarily to the cation component of the ionic liquid, 1-ethyl-3-methylimidazaolium. Most published information indicates the anion has little impact on the toxicity of the salt. However, dicyanamide (DCA) salts appear to be more toxic by a factor of 2.6 when compared to analogous mono-halide salts (i.e., such as the bromide or chloride) (Samori, 2007; Stolte, 2006).

7.3.1 Acute Oral

No experimental data found. TOPKAT modeling predicts an oral LD_{50} in rats of 6.2 grams per kilogram (g/kg) with high confidence. The 95 percent confidence interval is 1.0 g/kg to >10 g/kg.

*The cation for an ionic liquid is portrayed in the first set of brackets and the anion in the second set.

7.3.2 Subchronic Oral

No data found.

7.3.3 Chronic Oral

No experimental data found. TOPKAT modeling predicts a chronic LOAEL of 51.9 mg/kg with high confidence. The 95 percent confidence interval is 12.4 mg/kg to 217.3 mg/kg.

7.3.4 Acute Inhalation

No experimental data found. TOPKAT modeling predicts an acute inhalation LC_{50} in rats to be >10 g/m^3-h with high confidence. The 95 percent confidence interval is 5.0 grams per cubic meter per hour (g/m^3-h) to >10 g/m^3-h.

7.3.5 Subchronic Inhalation

No data found.

7.3.6 Chronic Inhalation

No data found.

7.3.7 Dermal/Ocular

No experimental data found. TOPKAT modeling predicts this compound will cause moderate to severe dermal irritation, but will not cause sensitization; TOPKAT also predicts this compound will be a severe eye irritant.

7.3.8 Development and Reproduction

No experimental data found. TOPKAT modeling predicts this compound will be a developmental or reproductive toxicant with high confidence.

7.3.9 Mutagenicity

No experimental data found. TOPKAT modeling predicts this compound will not be mutagenic in the Ames assay.

7.3.10 Carcinogenicity

No experimental data found. TOPKAT modeling provides mixed results but suggests a slightly less than even chance this compound will be a carcinogen.

7.3.11 Ecotoxicology

7.3.11.1 Fate and Transport

High water solubility and small K_{oc} values indicate this substance is a likely threat to ground water if released to the environment. EPI Suite modeling predicts that this compound will not readily biodegrade.

7.3.11.2 Ecotoxicity

No experimental data found. TOPKAT was unable to make a prediction on toxicity toward fathead minnows due to lack of a suitable model, however an EC_{50} of 246.6 mg/L was predicted for *Daphnia*.

7.4 1-Butyl-3-methylimidazolium dicyanamide, [BMIM][DCA]

Virtually no experimental toxicological data are available for this or structurally similar compounds. The information below pertains primarily to the cation component of the ionic liquid—BMIM. Most published information indicates the anion has little impact on the toxicity of the salt. However, DCA salts appear to be more toxic by a factor of 2.6 when compared to analogous mono-halide salts (i.e., such as the bromide or chloride; Samori et al., 2007; Stolte et al., 2006).

7.4.1 Acute Oral

No experimental data found. TOPKAT modeling estimates the oral LD_{50} in rats to be 7.8 g/kg, with low confidence. The 95 percent confidence interval for this value is 1.2 g/kg to greater than 10 g/kg.

Landry and coworkers evaluated the toxicity of the closely related compound, 1-butyl-3-methylimidazolium chloride (BMIC). Using the Approximate Lethal Dose Up/Down procedure, an LD_{50} of 550 mg/kg was determined in female rats. The 95 percent confidence interval on these data is 380.9 mg/kg to 1710 mg/kg. At the highest dose administered (2000 mg/kg), animals exhibited hypoactivity and abnormal posture prior to death within less than 24 hours after dose administration. Gross necropsy revealed discoloration of the intestines (Landry et al.,2005).

7.4.2 Subchronic Oral

No data found.

7.4.3 Chronic Oral

No experimental data found. TOPKAT modeling estimates a chronic LOAEL in rats of 59.4 mg/kg, with high confidence. The 95 percent confidence interval for this value is 14.4 mg/kg to 245.5 mg/kg.

7.4.4 Acute Inhalation

No experimental data found. TOPKAT modeling estimates an acute LC_{50} to be greater than 10 g/m^3-h with low confidence. The 95 percent confidence interval for this value exceeds 10 g/m^3-h at both the upper and lower limits.

7.4.5 Subchronic Inhalation

No data found.

7.4.6 Chronic Inhalation

No data found.

7.4.7 Dermal/Ocular

No experimental data found. TOPKAT modeling predicts that [BMIM] will be a moderate to severe skin irritant with a moderate degree of confidence but is not expected to be a sensitizer.

Landry and coworkers also evaluated the dermal toxicity of the surrogate BMIC. Following dermal administration of 2000 mg/kg in water, the only abnormalities noted in test animals were erythema and edema at the site of administration. Dermal tests were also conducted in dimethylformamide (DMF), with the result being that 2 of 5 males and 5 of 5 females died following administration of a 75 percent solution of BMIC. The dermal LD_{50} for BMIC in water was determined to be >2000 mg/kg in both sexes and when administered in DMF, >2000 mg/kg in males and between 800 and 2000 mg/kg for females. In the eye irritation study, undiluted BMIC was mildly irritating to the eyes. In the Local Lymph Node Assay (LLNA), BMIC exhibited potential for being a dermal sensitizer (Landry et al., 2005).

7.4.8 Development and Reproduction

No experimental data found. TOPKAT modeling predicts BMIM will be a developmental or reproductive toxicant with a high degree of confidence.

7.4.9 Cytotoxicity

Stepnowski and coworkers investigated the effect of ionic liquids on the human tumor cell line HeLa. For 1-butyl-3-methylimidazolium entities, the cytotoxicity was found to depend upon the nature of the anion; tetrafluoroborate having the lowest EC_{50} at 0.63 milligrams/liter(mg/L). An hormesis effect was observed for [BMIM][BF_4] after a 44-hour incubation (Stepnowski et al., 2004).

7.4.10 Mutagenicity

Xie and coworkers have demonstrated the ability of [BMIM][BF_4] to interact with DNA *in vitro*, presumably via an electrostatic mechanism. The authors were able to determine thermodynamic and kinetic parameters of binding and dissociation as part of the experiment. The Gibbs Free

Energy of surface binding was determined to be -26.4 kilojoules per mole (kJ/mol) at 298 K (Xie et al., 2008).

No descriptive toxicology data pertaining to mutagenesis was found. TOPKAT modeling indicates [BMIM] is not mutagenic, with high confidence.

Docherty and coworkers evaluated the related compound 1-butyl-3-methylimidazolium chloride (BMIC) as part of a study of mutagenicity in ionic liquids. While not strictly meeting the criteria for mutagenicity, BMIC did show some mutagenic potential at high doses, i.e. at 5 mg/plate and higher concentrations in *Salmonella typhimurium* strain TA98. Treatment with S9 fraction eliminated this tendency, which was not present either with or without S9 activation in the TA100 strain (Docherty et al., 2006).

7.4.11 Carcinogenicity

No experimental data found. TOPKAT modeling indicates [BMIM] is not likely to be carcinogenic.

7.4.12 Ecotoxicology

7.4.12.1 Fate and Transport.

Available information suggests that ionic liquids are highly water soluble and resistant to environmental degradation (Gathergood et al., 2004; Garcia et al., 2005, Samori et al., 2007) EPI Suite modeling predicts a water solubility for this compound of 1 x 10^6 mg/L, making it extremely water-soluble, with essentially no tendency to volatilize from water into the atmosphere (K_H = 4.00 x 10^{-9} atmosphere-cubic meter per mold (atm-m^3/mol), and with no tendency to biodegrade, confirmed experimentally by Gathergood and coworkers (Gathergood et al., 2004). The soil adsorption coefficient (K_{oc}) is calculated to be 330.4 liters per kilogram (L/kg), indicating its ability to adsorb to organic carbon is low. The bioconcentration factor is estimated at 3.162 L/kg body weight, indicating it is unlikely to accumulate in the food chain.

7.4.12.2 AquaticToxicity.

Samori and coworkers (Samori et al., 2007) compared the toxicity of the three ionic liquids [BMIM][BF_4], [MOEMIM][BF_4] (1-methoxyethyl-3-methylimidazolium tetrafluoroborate) and [MOEMIM][DCA] (1-methoxyethyl-3-methylimidazolium dicyanamide) in *Daphnia magna* and *Vibrio fischeri* (Microtox test). Mean values for the results of this experiment are given in the table below:

Table 3. Aquatic Toxicity of Selected Ionic Liquids

Compound	*Daphnia magna* (EC_{50} mg/L)	*Vibrio fischeri* (EC_{50} mg/L)
[BMIM][BF_4]	5.18	300
[MOEMIM][BF_4]	215	3196
[MOEMIM][DCA]	209	2406

The authors concluded from this limited data set that introduction of oxygen to the side chain of the imidazolium side chain could result in reduced toxicity. [BMIM][BF_4] is less toxic than long alkyl-

chain imidizolium salts, suggesting toxicity increases with hydrophobicity. Toxicity of [BMIM][BF_4] in *Daphnia magna* and *Vibrio fischeri* is comparable to that of chlorinated organic solvents but is more toxic than nonchlorinated organic solvents such as methanol, acetone, and acetonitirile (Samori et al., 2007).

Cho and coworkers investigated the toxicity of several ionic liquids, including [BMIM][Br] to the *Selenastrum capricornutum* algae. The susceptibility of algae to ionic liquids was found to vary according to chain length, with BMIM being comparable to the least toxic of the cations under evaluation. The toxicity of [BMIM][Br] was found to increase as the system incubation time increased from 48 to 96 hours (Cho et al., 2007). A hypothesis regarding the source of this increasing toxicity, at least when the tetrafluoroborate anion is present, is explored in the following paragraph.

In a follow-on to the study discussed in the preceding paragraph, Pham and coworkers investigated the impact of various ionic liquids on photosynthetic activity and growth rate in *Selenastrum capricornutum* algae. The ionic liquids [BMIM][BF_4] and [BMIM][Br] were chosen for these investigations. Both compounds inhibited growth of the algae at comparable concentrations, with EC_{50} values being 2138 micromolar (micromoles/liter, µM) for the bromide salt, and 3467 µM for the boron tetrafluoride salt. There were substantial differences in the EC_{50} values for inhibition of photosynthesis, however, with the EC_{50} being 23,998 µM for the bromide salt, and 3715 µM for the tetrafluoroborate salt. Also of note, there appears to be an aging phenomenon associated with these compounds, as freshly prepared solutions were much less potent in inducing cellular toxicity than solutions that had aged. This aging phenomenon is believed by the authors and several others cited in the article, to be the result of hydrolysis of the fluorinated anions with subsequent production of fluoride ion. Measurements made over the course of 9 days to assess the rate of fluoride ion formation resulted in the finding that approximately 14 mg/L-day fluoride was produced for a mixture that initially contained an unspecified amount of [BMIM][BF_4] (Pham et al., 2008).

Pretti and coworkers reported on testing of several ionic liquids in zebrafish (*Danio rerio*). [BMIM][DCA] was found to have a toxic concentration in excess of 100 mg/L, the limit test in this experiment. However, fish exposed to concentrations of all ionic liquids at concentrations in excess of 10 mg/L demonstrated reduction in general activity, loss of equilibrium, erratic swimming and stayed motionless at mid-water for prolonged periods when compared to controls (Pretti et al., 2008).

TOPKAT modeling in *Daphnia* estimates an EC_{50} of 617.7 mg/L with moderate confidence. The 95 percent confidence interval on this value is 113.9 mg/L to 3.3 g/L. Modeling in fathead minnow could not be conducted due to lack of a suitable model.

7.4.12.3 Plants.

Wang and colleagues investigated the effects of [BMIM][BF_4] on wheat seed germination and growth. At 0.4 millimolar (millimoles/liter, mM) (34.7 mg/L), no effects were observed in wheat germination or seedling shoot and root lengths, but germination was reduced to 38.0 percent of control in the presence of 4.4 mM (380 mg/L) [BMIM][BF_4]. Wheat seedlings and shoots were shortened at [BMIM][BF_4] concentrations above 0.9 mM. Activity of amylase increased in roots and shoots but decreased significantly in germinating seeds when [BMIM][BF_4] concentrations exceeded 1.8 mM. The authors concluded that concentrations of [BMIM][BF_4] exceeding 0.9 mM (156 mg/L) were toxic to wheat seedlings (Wang et al., 2009).

7.5 1-Butyl-1-methylpyrrolidinium dicyanamide, [BMP][DCA]

The information below pertains primarily to the cation component of the ionic liquid. Most published information indicates the anion has little impact on the toxicity of the salt. However, dicyanamide salts appear to be more toxic by a factor of 2.6 when compared to analogous mono-halide salts (i.e. such as the bromide or chloride;Stolte et al., 2006).

7.5.1 Acute Oral

No experimental data found. TOPKAT predicts the rat oral LD_{50} to be 1.1 g/kg with high confidence. The 95 percent confidence interval is 235.0 mg/kg to 5.5 g/kg.

7.5.2 Subchronic Oral

No data found

7.5.3 Chronic Oral

No experimental data found. TOPKAT estimates a chronic LOAEL of 7.8 mg/kg with high confidence. The 95 percent confidence interval is 2.4 mg/kg to 25.2 mg/kg.

7.5.4 Acute Inhalation

[BMP][DCA] is reported to be irritating to the respiratory system (Sigma-Aldrich, 2010). TOPKAT estimates the LC_{50} to be greater than 10.0 g/m^3-h with high confidence. The 95 percent confidence interval is 1.6 g/m^3-h to >10.0 g/m^3-h.

7.5.5 Subchronic Inhalation

No data found.

7.5.6 Chronic Inhalation

No data found.

7.5.7 Dermal/Ocular

Irritating to skin; may cause sensitization (Sigma-Aldrich, 2010). TOPKAT predicts this compound is likely to produce skin sensitization.

Irritating to eyes (Sigma-Aldrich, 2010). TOPKAT predicts this compound will likely cause mild ocular irritancy.

7.5.8 Development and Reproduction

No experimental data found. TOPKAT predicts this compound will not be a developmental or reproductive toxicant.

7.5.9 Mutagenicity

No experimental data. TOPKAT predicts this compound will not be mutagenic in the Ames test with high confidence.

7.5.11 Carcinogenicity

No experimental data. TOPKAT predicts this compound will not be carcinogenic with high confidence.

7.5.12 Ecotoxicology

7.5.12.1 Fate and Transport

No experimental data. EPI Suite predicts this compound will rapidly biodegrade and will not bioaccumulate.

7.5.12.2 Ecotoxicity.

No experimental data. TOPKAT estimates an EC_{50} for *Daphnia* of 254.4 mg/L and an LC_{50} in fathead minnow of 52.6 mg/L.

7.6 Dicyanamide, [DCA]

Dicyanamide is not a distinct compound in this work unit but is the anionic component of the three preceding cations in these ionic liquids. Because little data could be found for the ionic liquid salts, each component of the compounds must be considered separately, if for no other reason than QSAR modeling is impossible for salts. Also, in certain environments, it is possible that the cation and anion of the ionic liquid will dissociate and each component will have to be considered separately. The sodium salt of dicyanamide, dicyanoamide was also considered as part of this survey.

Stolte and coworkers examined the effects of the anion component of various ionic liquids on IPC-81 rat leukemia cells using the WST-1 (2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium monosodium salt) assay. The EC_{50} of the [BMIM][DCA] ionic liquid was 1400 µM, representing a 2.6-fold increase in toxicity over the chloride control (EC_{50} = 3600 µM; Stolte et al., 2006).

7.6.1 Acute Oral

No experimental data found. TOPKAT modeling estimates an oral LD_{50} in rat of 160.6 mg/kg with high confidence. The 95 percent confidence interval is 31.8 mg/kg to 808.8 mg/kg.

7.6.2 Subchronic Oral

No data found.

7.6.3 Chronic Oral

No experimental data found. TOPKAT modeling estimates a chronic LOAEL of 3.1 mg/kg with high confidence. The 95 percent confidence interval is 550.6 µg/kg to 17.7 mg/kg.

7.6.4 Acute Inhalation

No experimental data found. TOPKAT modeling estimates an acute LC_{50} in rats of 6.0 g/m^3-h with moderate confidence. The 95 percent confidence interval is 251.9 mg/m^3-h to >10 g/m^3-h.

7.6.5 Subchronic Inhalation

No data found.

7.6.6 Chronic Inhalation

No data found.

7.6.7 Dermal/Ocular

No experimental data found. TOPKAT modeling predicts DCA has a moderate probability of being a skin irritant and is likely to cause skin sensitization. In addition, TOPKAT modeling predicts DCA to be a moderate to severe eye irritant with moderate confidence.

7.6.8 Development and Reproduction

No experimental data found. TOPKAT modeling estimates DCA will be a developmental or reproductive toxicant with high confidence.

7.6.9 Mutagenicity

No experimental data found. TOPKAT modeling predicts DCA will not be mutagenic in the Ames assay.

7.6.10 Carcinogenicity

No experimental data found. TOPKAT modeling is mixed but suggests DCA is not likely to be a carcinogen.

7.6.11 Ecotoxicology

7.6.11.1 Fate and transport

With respect to fate and transport, EPI Suite 4.0 model predicts dicyanamide will be readily biodegraded with a timeframe of days to weeks.

7.6.11.2 Ecotoxicity

No experimental data found. TOPKAT modeling predicts an LD_{50} in fathead minnow of 70.7 µg/L and an EC_{50} in *Daphnia* of 922 g/L, both with low confidence. The 95 percent confidence intervals for these values are 1.4 µg/L to 3.5 mg/L and 15.6 g/L to greater than 1000 g/L, respectively.

7.7 1,2,4-trimethyl-1,4-diazabicyclo[2.2.2]octane dinitrate

7.7.1 Acute Oral

No experimental data found. TOPKAT modeling predicts an oral LD_{50} in rats of 1.1 g/kg body weight, with high confidence. The 95 percent confidence interval for this figure is 233.1 mg/kg to 5.5 g/kg.

7.7.2 Subchronic Oral

No data found.

7.7.3 Chronic Oral

No experimental data found. TOPKAT modeling predicts a LOAEL in rats of 2.2 mg/kg, with moderate confidence. The 95 percent confidence interval on this figure is 527.7 µg/kg to 8.9 mg/kg.

7.7.4 Acute Inhalation

No experimental data found. TOPKAT modeling predicts an LC_{50} in rats of 2.3 g/m^3-h, with high confidence. The 95 percent confidence interval is 172.3 mg/m^3-h to >10 g/m3-h.

7.7.5 Sub-Chronic Inhalation

No data found.

7.7.6 Chronic Inhalation

No data found.

7.7.7 Dermal/Ocular

No experimental data found. TOPKAT modeling predicts this compound will be neither a skin irritant nor sensitizer. However, it is predicted to be a severe ocular irritant.

7.7.8 Development and Reproduction

No experimental data found. TOPKAT modeling predicts this compound will be a developmental or reproductive toxicant with high confidence.

7.7.9 Mutagenicity

No experimental data found. TOPKAT modeling predicts this compound will not be mutagenic in the Ames *S. typhimurium* assay with high confidence.

7.7.10 Carcinogenicity

No experimental data found. TOPKAT modeling predicts this compound will not be carcinogenic.

7.7.11 Ecotoxicology

7.7.11.1 Fate and transport

Based upon high water solubility and low K_{ow} and K_{oc}, this compound is likely to transport through ground water and become a potential ingestion risk. EPI Suite modeling predicts it will not be readily biodegradable, with a persistence in the environment of several weeks. The calculated bioconcentration factor of 3.162 L/kg wet weight indicates it is unlikely to accumulate in the food chain.

7.7.11.2 Ecotoxicity

No experimental data found. TOPKAT modeling predicts an LC_{50} for Daphnia exceeding 1000 g/L, and an LC_{50} in fathead minnows of 211.8 mg/L. The 95 percent confidence intervals for these values are 182.1 g/L to >1000 g/L and 66.0 mg/L to 680.0 mg/L, respectively.

7.8 Tris-(2-nitratoethyl)methylammonium nitrate

Tris-(2-nitratoethyl)methylammonium nitrate is the only acyclic quaternary ammonium salt to be considered in this investigation. Compounds of this type have been reported to be more toxic than the imidazolium or pyrridolinium classes of ionic liquids (Pretti et al., 2006; 2009).

7.8.1 Acute Oral

No experimental data found. TOPKAT modeling predicts an oral LD_{50} in rats of 49.9 mg/kg, with low confidence. The 95 percent confidence interval on this value is 6.6 mg/kg to 380.3 mg/kg.

7.8.2 Subchronic Oral

No data found.

7.8.3 Chronic Oral

No experimental data found. TOPKAT modeling predicts a chronic LOAEL of 69.9 nanograms per kilogram (ng/kg) body weight in the rat, with low confidence. The 95 percent confidence interval on this value is 1.7 ng/kg to 2.8 micrograms per kilogram (µg/kg).

7.8.4 Acute Inhalation

No experimental data found. TOPKAT modeling predicts an acute inhalation LC_{50} of 1.7 mg/m^3-h, with low confidence. The 95 percent confidence interval on this value is 25.5 $\mu g/m^3$-h to 116.6 mg/m^3-h.

7.8.5 Sub-Chronic Inhalation

No data found.

7.8.6 Chronic Inhalation

No data found.

7.8.7 Dermal/Ocular

No experimental data found. TOPKAT modeling predicts this compound will not be either a skin irritant or sensitizer but does predict it will cause mild to severe ocular irritation, at a low degree of confidence.

7.8.8 Development and Reproduction

No experimental data found. TOPKAT modeling predicts this compound will be a developmental or reproductive toxicant with a moderate level of confidence.

7.8.9 Mutagenicity

No experimental data found. TOPKAT modeling predicts this compound will be mutagenic in the Ames *S. typhimurium* assay, with high confidence.

7.8.10 Carcinogenicity

No experimental data found. TOPKAT modeling predicts this compound will be carcinogenic, with moderate to high confidence.

7.8.11 Ecotoxicology

7.8.11.1 Fate and Transport

High water solubility and small K_{oc} values indicate this substance is a likely threat to ground water if released to the environment. EPI Suite predicts this compound will not be readily biodegradable, with persistence in the environment of weeks to months.

7.8.11.2 Ecotoxicity

No experimental data found. TOPKAT modeling predicts an LC_{50} in *Daphnia* of 557.0 µg/L at a low level of confidence. The 95 percent confidence interval on this value is 9.9 µg/L to 31.3 mg/L. For fathead minnow, the LC_{50} is estimated to be 6.0 g/L, with moderate confidence. The 95 percent confidence interval for this value is 783.3 mg/L to 46.1 g/L.

Table 4. Physical and Chemical Properties

Compound	Molar mass (g/mole)	Boiling Point (°C)	Aqueous solubility (mg/L) @ 25°C	log K_{ow}	log K_{oc}	Henry's Law Constant (atm-cubic meter per mole (m^3/mol) @ 25°C	Vapor pressure mmHg @ 25°C
Hydrazine	32.05[1]	113.5[4]	Miscible[4]	-2.07[6]	2[6]	Negligible	Negligible
1-ethyl-3-methylimidazole	111.19[2]	231.16[3]	1E+06[3]	-2.33[3]	0.035[3]	1.09E-08[3]	0.0736[3]
1-butyl-3-methylimidazole	139.25[2]	266.24[3]	1E+06[3]	-1.35[3]	0.58[3]	4.00E-09[3]	8.78E-03[3]
1-butyl-1-methylpyrrolidine	142.3[2]	373.92[3]	5.90E+04[3]	0.16[3] 2.58[4]	0.926[3] 2.23[3a]	4.79E-13[3]	2.48E-06[3]
Dicyanamide	67.05[1]	203.63[3]	3.80E+05[3]	-0.52[3]	1.42[3]	7.00E-08[3]	0.301[3]
Ammonium perchlorate	117.50[1]	na	2.2E+05[7]	na	na	Negligible	Negligible
1,2,4-trimethyl-1,4-diazabicyclo[2.2.2]octane dinitrate	158.29[1]	535.58[3]	5.86E+04[3]	0.09[3]	0.80[3]	7.74E-17[3]	2.18E-11[3]
tris-(2-nitratoethyl)-methylammonium nitrate	299.26[1]	505.64[3]	1E+06[3]	-2.94[3]	-0.79[3]	7.323E-17[3]	1.86E-10[3]

Legend:
na - not applicable; nd-no data
g/mole – grams per mole
°C – degrees Celsius
m^3/mol – cubic meter per mole
mmHg – millimeters per mercury

Notes:
[1] - Calculated from molecular formula and standard atomic weights.
[2] - Sigma-Aldrich MSDS, 2010
[3] - EPI Suite 4.0 estimate.
[3a] - EPI Suite 4.0 estimate using MCI method.
[4] - TOPKAT estimate.
[5] - O'Neil, 2006.
[6] - HSDB, 2009.
[7] - Dean, 1992.

Table 5. Toxicological Data

Compound	Acute (mg/kg)	Subacute (mg/kg-d)	Subchronic (mg/kg-d)	Chronic (mg/kg-d)	Mutagenicity	Carcinogenicity
Hydrazine	59[2] (mouse) 60[2] (rat)	nd	nd	nd	Positive[2]	Positive[2]
1-ethyl-3-methylimidazole	6200[1]	nd	nd	51.9[1]	Negative[1]	Indeterminate[1]
1-butyl-3-methylimidazole	7800[1]	nd	nd	59.4[1]	Negative[1]	Negative[1]
1-butyl-1-methylpyrrolidine	1.1[1] (rat)	nd	nd	7.8[1]	Negative[1]	Negative[1]
Dicyanamide	160.5[1]	nd	nd	3.1[1]	Negative[1]	Indeterminate[1]
Ammonium perchlorate	1900[3] (mouse, rabbit) 3310[3] (guinea pig) 4200[3] (rat)	nd	nd	nd	Negative	Thyroid tumors at high doses causing hyperplasia; possible promotor.
1,2,4-trimethyl-1,4-diazabicyclo[2.2.2]octane dinitrate	1100[1]	nd	nd	2.2[1]	Negative[1]	Negative[1]
tris-(2-nitratoethyl)-methylammonium nitrate	49.9[1]	nd	nd	7.0E-06[1]	Positive[1]	Positive[1]

Notes:
[1] - TOPKAT estimate.
[2] - HSDB, 2009.
[3] - CIDPL, 2009.

Table 6. Human Health Toxicity Assessment

Compound	Acute oral	Subchronic Oral	Acute Inhalation	Subchronic Inhalation	Cancer Probability	Comments
Hydrazine	High	High	High	High	High	Carcinogenic in animals.
1-ethyl-3-methylimidazole	Low	Unk	Low	Unk	Unk	
1-butyl-3-methylimidazole	Low	Low	Low	Low	Low	
1-butyl-1-methylpyrrolidine	Low	Unk	Low	Unk	Low	
Dicyanamide	Low	Unk	Low	Unk	Mod	Non-genotoxic mechanism possible for cancer.
Ammonium perchlorate	Low	Low	Low	Low	Low	
1,2,4-trimethyl-1,4-diazabicyclo[2.2.2]octane dinitrate	Mod	Unk	Low	Unk	Low	
tris-(2-nitratoethyl)-methylammonium nitrate	High	Unk	Low	Unk	Mod-High	All assessments based exclusively on modeling data.

Legend:
Unk-Unknown

Table 7. Ecotoxicology Assessment

Compound	Aquatic	Invertebrates	Plants	Mammals	Birds	Comments
Hydrazine	Mod-High	High	Mod	High	nd	
1-ethyl-3-methylimidazole	Unk	Unk	Unk	Unk	Unk	
1-butyl-3-methylimidazole	Low	Low	Low	Low	Unk	
1-butyl-1-methylpyrrolidine	Low	Low	Unk	Low	Unk	
Dicyanamide	Unk	Unk	Unk	Unk	Unk	
Ammonium perchlorate	Low	Low	Low	Low	Low	
1,2,4-trimethyl-1,4-diazabicyclo[2.2.2]octane dinitrate	Low	Low	Unk	Mod	Unk	
tris-(2-nitratoethyl)-methylammonium nitrate	High	High	Unk	Unk	Unk	

8 Discussion

8.1 General

Experimental physical and toxicological property information are lacking for essentially all of the compounds investigated in this report. The QSAR modeling to fill data gaps is hindered by the fact that all of these compounds are salts, and currently available QSAR models are not designed to evaluate salts; the cation and anion of the salt must be evaluated separately and a synthesis of their combined properties developed. This procedure has inherent difficulties and risks.

A comparison of the fate, transport, and toxicological properties of these five compounds is presented in Table 8—Fate and Transport Summary.

Table 8. Fate and Transport Summary

Compound	Solubility	Readily Biodegradable	Transport Potential	Bioaccumulation Potential
1-ethyl-3-methylimidazolim dicyanamide	High	No	High	Low
1-butyl-3-methylimidazolium dicyanamide	High	No	High	Low
1-butyl-1-methylpyrrolidinium dicyanamide	High	Yes	High	Low
1,2,4-trimethyl-1,4-diazabicyclo[2.2.2]octane dinitrate	High	No	High	Low
tris-(2-nitratoethyl)-methylammonium nitrate	High	No	High	Low

8.2 Regulations and Standards

No regulations or standards pertaining to these compounds were located.

8.3 Areas of Uncertainty

Lack of experimental data for these compounds is the greatest source of uncertainty. Because of this lack of data, projections must be made on the basis of QSAR data alone. While QSAR is a useful tool and can point toward areas of concern, it does not replace experimental work. Moreover, the database has few examples with similar moieties from which to draw model predictions, so many of these predictions are relatively low in confidence with few exceptions. Specific data gaps for each compound are discussed below.

A significant area of uncertainty is the contribution of the anionic component of the ionic liquid to the toxicity of the substance. Literature comments on this matter appear to be in conflict, with some

asserting that the anion has little impact on toxicity, while others indicate that there are significant differences. This uncertainty reinforces the need for direct experimental data for these substances.

8.3.1 1-Ethyl-3-methylimidazolium dicyanamide

No experimental acute oral toxicity or acute inhalation toxicity data are available for this compound. TOPKAT estimates indicate this compound is unlikely to be highly toxic when exposure occurs via the inhalation or oral ingestion routes. While generally indicating this compound is unlikely to be either mutagenic or carcinogenic, QSAR modeling suggests a possibility for reproductive or developmental toxicity.

Environmental toxicity is also an area of uncertainty for this substance and, therefore, concern. Physical properties of this compound indicate that it will be highly water soluble with no tendency to partition into the atmosphere and little affinity for organic carbon. In addition, EPI Suite modeling indicates it is not readily biodegradable and, therefore, will be persistent in the environment.

There appears to be a significant exposure risk relating to this chemical should it be released to the environment and enter the ground water. Although acute toxicity is low, questions about long-term effects, including reproductive effects, are unknown.

8.3.2 1-Butyl-3-methylimidazolium dicyanamide

While review of the literature suggests the 1-butyl-3-methylimidazolium cation is not likely to be particularly toxic, there are some areas of concern. Firstly, there is essentially no toxicity information available on the [BMIM][DCA] salt combination. All conclusions must be derived from a synthesis of results derived independently for the cation and the anion. Also, a significant amount of the information was derived through QSAR approaches, a minimum of which should be validated via experiment. Also, necropsy of animals treated with the closely related compound BMIC (1-butyl-3-methylimidazolium chloride) indicated the presence of discoloration to the intestines during necropsy, suggestive of the potential for attack of the digestive system and internal bleeding.

Perhaps the greatest uncertainty arises from the observation that this compound is highly water soluble, binds poorly to organic carbon, does not partition into the atmosphere, and according to EPI Suite modeling is highly resistant to biodegradation. Combined, these characteristics indicate that once released into the environment, at least the [BMIM] cationic component is likely to persist and could cause human exposure concerns.

In two of the aquatic studies discussed in paragraph 7.4, it was observed that the toxicity of [BMIM] salts—either the bromide or tetrafluoroborate salts—increased with age. In one case, it is proposed that the increasing toxicity is due to hydrolysis of the tetrafluoroborate anion with the associated production of hydrofluoric acid. While plausible for the case with the tetrafluoroborate (BF_4^-) anion, this is not a possible explanation with regard to the bromide anion since bromide is the conjugate of a strong acid. It seems more likely that the effects observed should be attributed directly to fluoride ion, not to hydrofluoric acid (HF).

8.3.3 1-Butyl-1-methylpyrridolinium dicyanamide

Based upon QSAR modeling, this compound appears to be the most environmentally benign of the three dicyanamide ionic liquids, but lacks experimental confirmation of QSAR predictions. Its acute toxicity is low, and QSAR modeling predicts this compound is not a developmental or reproductive toxicant, is not expected to be mutagenic in the Ames assay, and is not expected to be carcinogenic.

From a fate and transport perspective, water solubility remains high with little affinity for organic carbon. Like the two previous compounds, some concerns exist should this compound enter ground water, but toxicity towards aquatic species appears to be low.

If selected for further development, *in vitro* testing to validate the modeling predictions, as well as an acute oral toxicity study should be conducted.

8.3.4 1,2,4-Trimethyl-1,4-diazabicyclo[2.2.2]octane dinitrate

No experimental acute oral toxicity or acute inhalation toxicity data are available for this compound. TOPKAT estimates indicate this compound is unlikely to be highly toxic when exposure occurs via the inhalation or oral ingestion routes. While generally indicating, this compound is unlikely to be either mutagenic or carcinogenic; QSAR modeling suggests a possibility for reproductive or developmental toxicity.

Based upon high water solubility and low K_{ow} and K_{oc}, this compound is likely to transport through ground water and become a potential ingestion risk. EPI Suite modeling predicts it will not be readily biodegradable, with a persistence in the environment of several weeks. The calculated bioconcentration factor of 3.162 L/kg wet weight indicates it is unlikely to accumulate in the food chain.

Because all of the values cited in this profile are based on QSAR modeling and not experiment, they are all subject to uncertainty. If development of this compound is to continue, comprehensive collection of experimental data should be undertaken.

8.3.5 Tris-(2-nitratoethyl)methylammonium nitrate

This compound is the only aliphatic quaternary ammonium compound in the group. The limited toxicology data available suggest that compounds of this type have higher toxicity than the imidazolium, methylpyrridinium, and other ionic salts (Pretti, 2006; 2009). Nitrate esters of similar structure have been proposed as a new class of non-steroidal anti-inflammatory drugs (NSAIDs, Serkov and Bezuglov, 2009a; 2009b) that potentially effect blood pressure and physiological homeostasis, and this possibility is worthy of evaluation for this compound, but there are no data at present to indicate whether or not this represents a serious health risk. As with all of the other compounds considered under this work unit, there are little to no experimental toxicological data available.

The TOPKAT estimate for acute toxicity is low; however, the estimate for chronic toxicity is high. Because of poor data fit with the modeling program, the quality of both of these estimates is low

and should be regarded with some skepticism. There is moderate confidence that this compound will be a developmental or reproductive toxicant, and predictions for mutagenicity and carcinogenicity are both positive with high confidence.

Like the other ionic liquids in this work unit, aqueous solubility for this compound is high, with little affinity to organic carbon. Prospects for biodegradability are poor, with moderate toxicity towards invertebrates at the bottom of the aquatic food chain.

If development of this compound is to continue, comprehensive collection of experimental data should be undertaken.

9 Recommendations

In view of the general lack of experimental data, collection of basic information with respect to both physical/chemical and toxicological properties should be undertaken for compounds selected for further development. Physical/chemical properties that should be experimentally confirmed evaluated include water solubility, octanol-water partition coefficients, and biodegradation/ persistence.

A battery of *in vitro* tests (see Table 9, below) is also recommended to evaluate/validate the QSAR predictions that these compounds are developmental or reproductive toxicants, mutagenic, or carcinogenic. Depending upon the outcome of these initial *in vitro* tests, additional testing may be required.

Table 9. Recommended Toxicology Testing

Compound	Test				
	SCE	CHO	Micronucleus	Microtox	Ames
1-ethyl-3-methylimidazolium dicyanamide	Yes	Yes	Yes	No	No
1-butyl-3-methylimidazolium dicyanamide	Yes	Yes	Yes	Yes	Yes
1-butyl-1-methylpyrrolidinium dicyanamide	No	No	No	Yes	Yes
1,2,4-trimethyl-1,4-diazabicyclo[2.2.2]octane dinitrate	Yes	Yes	Yes	Yes	No
tris-(2-nitratoethyl)-methylammonium nitrate	Yes	Yes	Yes	Yes	Yes

Appendix A

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Appendix B

The TOPKAT System

B-1 Introduction

The TOPKAT consists of a basic program that controls data entry, toxicity estimate calculations and search functions for its model databases. An individual model is provided for each toxicologic endpoint, (e.g., LD_{50}, LOAEL, mutagenicity, carcinogenicity (male rat), carcinogenicity (female rat), and so forth).

A module may contain more than one database, each comprised of compounds in a certain structural class (e.g., multiple benzenes, alicyclics, and so forth). All predictive equations and validation procedures applied to a given query compound are based on the database for the structural class to which the query belongs. Thus, from each database a separate model is developed for making estimates of the relevant endpoint for chemicals in that structural class.

Each database contains a substantial number of compounds, often between 100 and 300, and an indication for reach compound of the actual toxicity, carcinogenicity, and so forth, from laboratory data, and whether it was used in generating the model. If it was used in model generation, the toxicity prediction for the compound, generated by the model, is also given. Generally, a small number of compounds are omitted from model generation as outliers or as wielding an undue influence on the model. Typically, the models yield an accuracy of about 95 percent for compounds in the model's database and compounds falling within the model's "optimum prediction space" (OPS).

The TOPKAT predicts the toxicity of a chemical structure based on statistically derived structure-activity relationships (SAR). The models are discrete molecular descriptors that identify functional groups present on a molecule and other parameters that can be used to quantify attributes of a particular structure. Standard databases were evaluated to obtain experimental values as input to the SAR equations.

For toxicity endpoints displaying continuous values, such as rat oral acute LD_{50} and rat chronic LOAEL, the system uses linear multiple regression equations, and the predictions represent estimates in dose units (mg/kg). For dichotomous endpoints, such as carcinogenicity and Ames mutagenicity, the models use two-group linear discriminant functions, and the output represents a probability, from 0 to 1, of a positive outcome for that endpoint. TOPKAT considers probability estimates from 0.3 to 0.7 to be indeterminate.

B-2 Carcinogenicity Endpoints

Carcinogenicity endpoints were estimated for four animal models (male rat, female rat, male mouse, and female mouse). Each TOPKAT-predictive model is developed from its own separate database and is limited to one of the foregoing animal models. Each covers a wide range of organic compounds, both aliphatic and aromatic. Results for a given compound sometimes vary from one animal model to another, even when confidence in the estimate is high or moderate.

Probable responses in rabbit eye and skin irritation tests (Draize) estimate probability of a severe response and also probability of a negative response. These two probabilities are then combined into an overall estimate (e.g., mild/moderate, severe, less than severe, or 'not negative').

B-3 Software

TOPKAT software includes an extensive procedure for internal validation of the estimate. The system checks to see if all substructures that comprise the query compound are represented among the compounds included in the database. The query compound is characterized by many descriptors, and the resulting multivariate description, or position in a multidimensional space, is automatically checked against the multidimensional OPS of the model. Generally, the SAR predictions generated by TOPKAT show a high probability of being accurate when all substructures are covered and the compound falls within the calculated OPS.

In all cases where actual data are not available, the user attempts to determine what level of confidence should be placed in the estimate. For estimates generated by the more recent versions of the software, the determinations provided by the built-in validation procedures are of first importance. Other factors used in developing suggestions of high medium or low confidence include, without being limited to the following:

- Whether all major structural features of the query compound are well represented in the model's database. At a certain level this results in an automatic warning from the software.
- Whether there are in the database a number of compounds that are judged by the software to be electrotopologically close to the query compound.
- Whether these near-by compounds are estimated accurately by the model and tend to present toxicity levels similar to that estimated for the query compound.
- Whether the model's database is reasonably large.
- Whether the compounds in the model's database are in general estimated with high accuracy.

For many estimates, meaningful results are not obtained. This usually results from the location of the query compound outside of the model's OPS and not within an acceptable distance from the OPS, or, alternatively, the presence in the query compound of a molecular fragment not adequately represented in the model's database.

For QSAR results, such as those discussed here, varying degrees of uncertainty always exist. It is common for about 80-90 percent of compounds not present in the pertinent database (but within the OPS of the model) to be predicted within a factor of five of the experimental value. Quantitative predictions, including mouse LC_{50}, rat LD_{50}, and rat chronic LOAEL, are accompanied by a 95 percent confidence range; these typically encompass values within a factor of four or five in either direction.